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RESTORATION OF VIABILITY OF IRRADIATED YEAST CELLS

[This is a translation of an article written by V. I. Korogodin and T. S. Malyutina in Priroda (Nature), No 10, 1959, pages 82-85.]

The elimination of harmful biological effects of ionizing radiations is the most important problem of modern radiobiology. The recovery of an irradiated multicellular organism can take place as a result of substitution of cells which perished following irradiation by the nondamaged elements of its organs and tissues as well as through the regeneration of the damaged cells themselves. Therefore, the success in working out methods of combatting radiation injuries depends on the extent of our knowledge of the role of these two processes in the pathogenesis of radiation sickness, and on the ability of influencing each of them.

The role of proliferation of nondamaged cells in the recovery of irradiated animals is well known. This phenomenon is already being utilized in the treatment of experimental radiation affection by means of injection of homogenates of the spleen or bone marrow. On the other hand, the possibility of restoring the viability of cells subjected to ionizing radiation has been subjected to doubt, up to recently.(1) However, some indications of such a possibility appeared in the radiobiological literature as far back as 40 years. G. A. Nadson, in particular, in his classical work devoted to the radiobiology of yeast organisms, notes that cells subjected to the action of radium preparations are able "to recover" when they are transferred to a fresh nutritional medium.(2) This observation has a principal significance: if the fate of irradiated cells depends not only on dose received, but also on the condition of their existence following irradiation, then the study of this phenomenon may put in the hands of radiobiologists a powerful weapon for

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(1) See Report of the United Nations Scientific Conference on the Effects of Atomic Radiation, New York, 1959.

(2) See G. A. Nadson "Herald of Roentgenology and Radiology", Vol 1, 1920, Nos 1-2, page 46

combatting radiation effect by means of action on its primary processes, which are taking place in the very cells of the irradiated organism. Unfortunately, the studies of G. A. Nadson, which were basically of a qualitative character, did not show convincingly whether the post-radiation cell which he had described was the rule or only the exception.

Ten years ago Sherman and Chase described the survival enhancement of yeast cells irradiated with X-rays after they had been inoculated on a nutritive medium during definite periods following irradiation. However, these authors explained their observations not by the regeneration of cells damaged by irradiation but as a proliferation of part of the population which had preserved its viability, at the expense of the waste products of cells which had been irreversibly damaged by radiation.(3)

In order to definitely solve the problem of the city of cells for post-radiation regeneration, it was necessary to verify experimentally the correctness of the Sherman and Chase hypothesis. One of the authors of the present article, not having found pertinent data in the literature, carried out a series of experiments on post-radiation regeneration on diploid yeast organisms. In these experiments he succeeded in reproducing the phenomenon described by Sherman and Chase, of the viability increase of irradiated yeast cells upon their preservation for 24 hours, and longer, in a non-nutritive medium, as well as in demonstrating the absence of a substantial lysis and proliferation of irradiated cells under these conditions.(1) However, these arguments in favor of the existence of the regeneration effect belong to the realm of indirect proof, and therefore cannot be considered to be sufficiently convincing.

Recently, we established that a considerable increase in the survival of diploid yeast cells, during the period of post-radiation mitotic rest, takes place also after their irradiation with 120-150 cr [Curie-Roentgen] doses, which causes the inactivation of up to 99.90 - 99.99 percent of the cells. The death of cells following irradiation with

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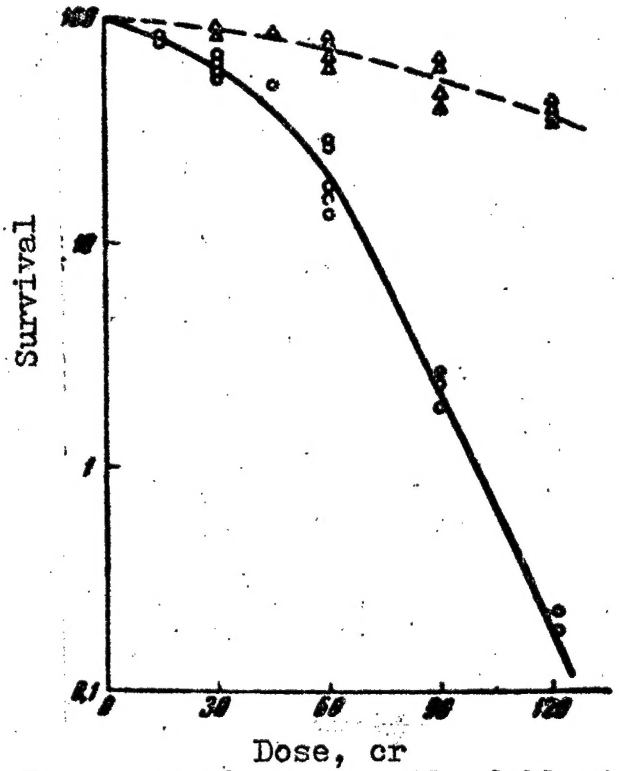
(3) See F. G. Sherman; N. B. Chase, J. Cell. Comp. Physiol, Vol 33, 1949, page 17

(1) See V. I. Korogodin, Biofizika [Biophysics], 1958, Vol III, No 6, page 703; V. I. Korogodin, O. V. Malinovskiy, N. A. Poryadkova, N. A. Izmarov, Tsitologiya [Cytology], Vol I, No 3, 1959, page 288

such doses takes place, basically, after one gemmation, and only rarely are there microcolonies observed to consist of four to five cells. However, when yeasts irradiated with such high doses remain in a non-nutritive medium 24 to 48 hours their survival capacity increases 200- to 1000-fold, and the inactivation of the nonregenerated part of the population takes place, in this case, after several cycles of proliferation. The survival curve (unbroken line) and the regeneation curve (dotted line) of diploid yeast cells which were the subject of our study, are shown in the figure.



Survival curves of yeast cells following irradiation with gammarays; unbroken line -- inoculation on a nutritive medium directly following irradiation; dotted line -- inoculation on nutritive medium within 24-48 hours following irradiation (regenration curve)

The effect of regeneration of yeast cells irradiated with doses causing such high mortality enabled us to verify the existence of this phenomenon by means of a direct experiment. As in previous experiments, the substances

studied were diploid yeasts -- Saccharomyces vini, strain Megri-139-B. The yeast suspension containing about 500 thousand cells per milliliter was irradiated with gamma rays of radioactive cobalt of 123-cr dose, which causes inactivation of 99.8 percent of the cells. Immediately following irradiation, the suspension was diluted 10 and 10,000 times with sterile, water-supply water. From the first dilution containing about 50 thousand cells per milliliter, an inoculation on Petri dishes was made in order to determine survival immediately after irradiation; the other dilution containing only about 50 cells per ml was poured out into 30 to 40 test tubes, one ml per tube. One half of these portions were inoculated on a nutritive medium immediately following their preparation in order to determine the frequency of the presence of cells in these protions capable of continuous proliferation immediately following irradiation. The remaining half of these portions was inoculated on a nutritive medium 48 hours after irradiation in order to determine the size of the regeneration effect; the latter was also determined in the suspension which had not been divided into portions and which had been preserved under identical conditions (in a thermostat at 30°). The survival capacity was determined by means of counting the macrocolonies in the Petri dishes after a four days' incubation at 30°. Nonirradiated yeast cells served as control. The data are given in the Table.

The results obtained show the following: after irradiation of yeast suspension containing about 500 thousand cells per ml with a 123-cr dose, the survival capacity was about 0.2 percent. When the irradiated suspension was diluted 10,000 times and divided into 1-ml portions, each such portion contained about 50 cells. Since 99.8 percent of these were not capable of forming macrocolonies immediately after irradiation, only in one or two out of ten portions could one cell be found which had not lost its capacity for proliferation following irradiation.

Table

Proof of the existence of the phenomenon of post-radiation regeneration of diploid yeast cells (dose 123 cr):

Groups	Experiment of 18 Nov 1959		Experiment 4 Mar 1959	
	Number of colonies	percent	No of colonies	percent
Control (dilution 1:10,000)	60.7 ± 1.3	100	44.6 ± 1.0	100
Immediately after irradiation (dilution 1:10)	141.0 ± 20.6	0.23	81.0 ± 8.7	0.18
Immediately after irradiation, portions of one ml (dil 1:10,000)	In 3 dishes out of 15 one colony grew in each (2:10)		In 2 out of 20 dishes one colony grew in each (1:10)	
Within 48 hours following irradiation, portions of one ml, (dilution 1:10,000)	In all 13 dishes 25.8 ± 7.1 colonies grew	42.5	In all 19 dishes 16.6 ± 3.5 colonies grew	37.2

Had the regeneration effect been an artefact, i. e., a phenomenon produced artificialy through treatment, and had the survival increase of irradiated yeast cells following their incubation in a non-nutritive medium been due to the proliferation of noninactivated individual cells, as was assumed by Sherman and Chase, we would have recorded the presence of this effect only in three to seven portions of the 32 studied. Actually, the marked increase in survival (200-fold) was recorded in all 32 portions of the irradiated suspension, of which at least 25 did not contain a single cell capable of continuous proliferation immediately following irradiation. In this connection the rate of regeneration in isolated tests proved to be identical with that of the irradiated suspension which had not been divided into portions (compare table and figure).

Thus, the post-radiation regeneration of diploid yeast cells irradiated with large doses of gamma rays proceeded with identical intensity in all tests which we had studied, independently of whether these tests contained cells noninactivated as the result of irradiation. This fact conclusively proves that the increased survival capacity of irradiated diploid yeast cells during their post-radiation

regeneration is not the privilege of diploid yeast cells only, but is characteristic of many unicellular organisms as well as cells of multicellular organisms.(1) This enabled V. I. Korogodin and N. V. Luchnik to suggest a working hypothesis, according to which the primary radiobiological changes in cells may have a potential character and a reversible form, and that the degree of their realization depends on the conditions of existence of these cells following irradiation. One may assume that the study of the nature of these potential injuries and development of methods directed toward the acceleration of their reduction will play a substantial role in combatting the harmful biological sequelae of the action of ionizing radiations.

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(1) A. W. Pratt, W. S. Moos, M. Eden, J. Nat. Cancer Inst, Vol 15, 1955, No 4, page 1039. See E. Ya. Grayevskiy Ye. G. Zinov'yeva, Doklady Akad Nauk SSSR [Reports of the Acad Sci USSR], Vol 121, 1958, No 5, page 837; N. V. Luchnik, L. S. Tsarapkin, ibid. Vol 124, 1959, No 1, page 213.

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